A Peroxisome Proliferator-Activated Receptor γ Agonist Influenced Daily Profile of Energy Expenditure in Genetically Obese Diabetic Rats

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ABSTRACT—Otsuka Long Evans Tokushima Fatty (OLETF) rats were developed as a model of non-insulin-dependent diabetes mellitus (NIDDM) with mild obesity. We reported that the daily profiles of energy expenditure associated with two peaks (one between 05:00 and 08:00 and the other between 20:00 and 22:00) were observed at 8 weeks of age (without NIDDM), while these two peaks disappeared at 24 weeks of age with NIDDM. As a new anti-diabetic drug, a peroxisome proliferator-activated receptor γ agonist pioglitazone hydrochloride has been developed, we examined whether pioglitazone normalized daily profiles of energy expenditure at 24 weeks of age. A control diet and pioglitazone (0.1%)-containing diet were fed from 6 weeks of age. The two peaks of daily profiles of energy expenditure, which disappeared in OLETF rats with the control diet at 24 weeks of age, were reproduced by administration of pioglitazone. The respiratory quotient was lower and fat derived energy used for combustion was increased by pioglitazone at both ages. The body weight, daily food intake, plasma levels of fat, insulin, leptin and the wet weight of visceral fat were not influenced, but the levels of blood hemoglobin A1c and plasma tumor necrosis factor α were decreased by pioglitazone. Administration of pioglitazone improved daily profiles of energy expenditure via affecting glucose and fat metabolisms.

Keywords: Energy expenditure, Non-insulin-dependent diabetes mellitus, Obesity, Peroxisome proliferator-activated receptor γ
in OLETF rats at 24 weeks of age after the manifestation of NIDDM, while these peaks were still substantial in normal control LETO rats at 24 weeks of age (5, 6). The profile of energy expenditure in OLETF rats with NIDDM at 24 weeks of age was also different from that in streptozotocin-induced insulin-dependent diabetes mellitus (IDDM) LETO rats, which constituted one peak (6).

It has been well known that obesity is highly correlated with insulin resistance and diabetes in experimental animals and humans. Reduction of body weight by either calorie-restriction, administration of α-glucosidase inhibitor or exercise, postponed the manifestation of NIDDM in OLETF rats (7–9). On the other hand, we recently found (10) that administration of a peroxisome proliferator-activated receptorγ (PPARγ) ligand pioglitazone did not affect the gain of body weight in OLETF rats; nevertheless, the plasma level of hemoglobin (Hb)A1c was significantly decreased, although it was still higher than the normal LETO rats. PPARγ agonists have been shown as novel class of anti-diabetic drugs that exhibit insulin-sensitizing activities (11) and also influence fat metabolism (12–14).

In the present study, the effects of a PPARγ agonist pioglitazone on daily profiles of energy expenditure were determined at 8 and 24 weeks of age (before and after the manifestation of NIDDM in OLETF rats with normal parameters) (1), and the sources of energy used for combustion were estimated on the basis of the respiratory quotient (RQ). The plasma and blood parameters of glucose and fat metabolisms as well as leptin and tumor necrosis factor (TNF)-α were measured by radioimmunoassay (19) with rat insulin as a standard. Plasma leptin was measured as previously reported (21, 22). Acrophase represents the time that shows the highest value of the fitting curve, mesor indicates the time-adjusted mean value, and amplitude is the amplitude of the cosine curves. A P value <0.01 was considered to signify a good fit.

MATERIALS AND METHODS

Animals and diets
The protocol was reviewed and approved by the appropriate committee of the Tokyo Metropolitan Institute of Gerontology. Male OLETF rats were obtained at 4 weeks of age from Otsuka Research Institute, Tokushima. Rats were maintained in individual cages in an air-conditioned room at 21°C with a 12-h light/12-h dark photocycle (08:00–20:00), at the Tokyo Metropolitan Institute of Gerontology.

Eight OLETF rats were fed a pioglitazone-containing diet (0.1%) from 6 weeks of age in OLETF rats. The control rats were fed a commercial rat chow (CRF-1; Oriental Co., Japan Inc., Tokyo) ad libitum (n = 8). The changes in body weights were recorded, and the mean value of daily food intake at 24 weeks of age was estimated.

OGTT
OGTT was conducted at 23 weeks of age. Glucose (2 g/kg) solution was given using orogastric tubing after overnight fasting, and plasma levels of glucose were measured before and at 30, 60 and 120 min after glucose ingestion.

Metabolic study and determinations
The metabolic study was conducted at 8 and 24 weeks of age because NIDDM manifests at 18 weeks of age in OLETF rats without any treatment (1) and the daily profile of energy expenditure at 8 weeks of age in OLETF rats was normal (5, 6).

Oxygen consumption and carbon dioxide production in expired air were measured continuously with an automatic O2–CO2 analyzer (Model IH26; NEC Medical Systems Co., Ltd., Tokyo). Energy expenditures per hour and per day were calculated. The sources of energy used for combustion during energy metabolism were estimated on the basis of the RQ during the metabolic study (15).

Blood and plasma biochemistry and tissue weight
After the completion of metabolic study, at 25 weeks of age, the rats were sacrificed by guillotine between 09:00 and 11:00 under conditions of no prior food-deprivation. The blood glucose and HbA1c concentrations were measured immediately (16). The rest of the blood was mixed with EDTA and centrifuged at 1500 × g for 15 min at 4°C, and the plasma was frozen at –30°C to measure biochemical parameters. The liver, pancreas and whole visceral fat tissues (the sums of mesenteric fat epididymal fat and peri-nephric fat) were removed and weighed. The pancreas was fixed with 10% formalin and examined by HE staining.

The plasma cholesterol and triglyceride were measured by enzymatic analysis (17, 18). Plasma insulin was measured by radioimmunoassay (19) with rat insulin as a standard. Plasma leptin was measured as previously reported (ELISA Rat Leptin Kit; Morinaga Institute of Biological Science, Yokohama) with rat leptin as a standard. Plasma TNF-α concentrations were measured as described (20).

Statistical analyses
Values are expressed as means ± S.E.M. Results were analyzed by one-way analysis of variance (ANOVA) or multiple analysis of variance (MANOVA) with repeated measures, followed by Fisher’s Least Significant Difference. A P value <0.05 was considered significant.

The daily profiles of energy expenditure derived from carbohydrate and fat were each analyzed by the cosinor method. A 24-h cosine curve was fitted to the data series (21, 22). Acrophase represents the time that shows the highest value of the fitting curve, mesor indicates the time-adjusted mean value, and amplitude is the amplitude of the cosine curves. A P value <0.01 was considered to signify a good fit.
RESULTS

Changes in body weight, food intake, organ weights and histology of the pancreas

There were no differences in terms of gain of body weight between OLETF rats with or without pioglitazone (695 ± 8 g for control, 716 ± 17 for pioglitazone, mean ± S.E.M.). The mean weights of rat chow consumed/day at 24 weeks of age were 27 – 29 g/day for control diet (CRF-1) and 28 – 29 g/day for pioglitazone-containing diet. The wet weights of liver, pancreas and total visceral fat, as well as the histological findings of the pancreas were not significantly different between treatments (not shown).

OGTT

The glucose tolerance curves were not significantly different between treatments, when analyzed by repeated measures MANOVA (F = 1.67, P>0.2 for treatment; F = 58.7, P<0.0001 for time; and F = 0.94, P>0.4 for interaction of strain and time). However, fasting blood glucose levels in OLETF treated with pioglitazone was significantly lower than those treated with the control diet, when analyzed by ANOVA (0.73 ± 0.03 mM for controls vs 0.60 ± 0.02 for pioglitazone, means ± S.E.M.).

Daily profile of energy expenditure

Energy consumption profiles over 24-h test periods at 8 weeks of age are shown in Fig. 1, upper panel. There were no significant differences between treatments. Two peaks, one between 05:00 and 08:00 and the other between 20:00 and 22:00, were observed. These patterns were similar to that observed in normal LETO rats (5, 6).

At 24 weeks of age, the two peaks were not apparent in control OLETF rats (Fig. 1, lower panel). In contrast, the two peaks were substantial during 06:00 – 08:00 and 16:00 – 24:00 in OLETF rats treated with pioglitazone. These two lines were significantly different, when analyzed by MANOVA with repeated measures.

The sources of energy used for combustion were estimated on the basis of RQ. The circadian changes in carbohydrate and fat metabolism were analyzed by the cosinor method. Energy derived from carbohydrate constituted the rhythm, whereas energy derived from fat did not constitute the rhythm between treatments at both 8 and 24 weeks of age. The curves of energy derived from carbohydrate were not significantly different between treatments. Figure 2 showed the results at 24 weeks of age.

Energy metabolism/day

The total energy expenditure (kJ·day⁻¹·kg⁻¹ body weight) was not significantly different between treatments, although the mean value in pioglitazone-treated OLETF rats tended to be higher than controls at 24 weeks of age (P = 0.16) (Table 1). The mean RQ was significantly lower in pioglitazone-treated OLETF rats compared with controls at both 8 and 24 weeks of age. Therefore, energy derived from fat was significantly increased by pioglitazone-treatment at both ages. The energy derived from carbohydrate was not significantly different between treatments at 24 weeks of age, while it was significantly lower at 8 weeks of age.

Blood and plasma chemistry

The plasma level of TNF-α was significantly decreased by pioglitazone treatment (9.7 ± 2.1 for control vs 3.2 ± 0.9 for pioglitazone, means ± S.E.M.), and reached to the
normal level (5.1 ± 1.8 nM, means ± S.E.M., n = 8 for normal LETO rats) (10). The levels of HbA1c were also decreased (3.53 ± 0.17% for controls vs 3.08 ± 0.09 for pioglitazone, means ± S.E.M.), although these were not fully returned to the normal levels (2.65 ± 0.03, means ± S.E.M., n = 8 for normal LETO rats) (10). However, other parameters, blood glucose (not fasting), insulin, triglyceride, cholesterol and leptin levels, were not significantly influenced by pioglitazone.

Table 1. Daily energy expenditure and sources of energy and RQ at 8 and 24 weeks of age

<table>
<thead>
<tr>
<th>Age</th>
<th>Control</th>
<th>Pioglitazone</th>
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<tbody>
<tr>
<td>8 weeks of age</td>
<td></td>
<td></td>
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<tr>
<td>Energy expenditure (kJ · day⁻¹ · kg⁻¹ body weight)</td>
<td>723.5 ± 16.2</td>
<td>723.7 ± 20.7</td>
</tr>
<tr>
<td>Energy derived from fat</td>
<td>51.9 ± 5.1</td>
<td>167.3 ± 8.2*</td>
</tr>
<tr>
<td>Energy derived from carbohydrate</td>
<td>670.2 ± 16.6</td>
<td>557.3 ± 14.1*</td>
</tr>
<tr>
<td>RQ (%)</td>
<td>0.98 ± 0.01</td>
<td>0.93 ± 0.02*</td>
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<tr>
<td>24 weeks of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy expenditure (kJ · day⁻¹ · kg⁻¹ body weight)</td>
<td>347.5 ± 9.5</td>
<td>372.9 ± 14.3</td>
</tr>
<tr>
<td>Energy derived from fat</td>
<td>55.8 ± 6.8</td>
<td>93.4 ± 24.9*</td>
</tr>
<tr>
<td>Energy derived from carbohydrate</td>
<td>292.7 ± 10.2</td>
<td>278.9 ± 14.6</td>
</tr>
<tr>
<td>RQ (%)</td>
<td>0.95 ± 0.01</td>
<td>0.92 ± 0.01*</td>
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</table>

Values are the mean ± S.E.M., n = 8 for each group. Energy derived from fat and carbohydrate were estimated using RQ values. *Significantly different from the corresponding control values, P<0.05.

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Fig. 2. Diurnal changes of fat metabolism and circadian changes of carbohydrate metabolism at 24 weeks of age with (left panel) or without pioglitazone (control) (right panel). There were no significant differences in energy expenditure derived from carbohydrate metabolism between control and pioglitazone-treated rats. The significant circadian rhythm was observed in carbohydrate metabolism both in control and pioglitazone-treated rats. The acrophases of carbohydrate and total energy expenditure were similar in range in both the control and pioglitazone-treated rats. Concerning fat metabolism, no significant circadian rhythm was detected in both control and pioglitazone-administered rats.
DISCUSSION

The daily profiles of energy expenditure in OLETF rats treated with control diet at 8 weeks (before the manifestation of NIDDM) and 24 weeks (after the manifestation of NIDDM) were different. The two peaks during 05:00–08:00 and 20:00–22:00 were observed at 8 weeks of age, but these peaks disappeared at 24 weeks of age. As we have not determined daily profiles of energy expenditure in another strain with NIDDM, it is unknown whether the disappearance of two peaks is a phenomenon specific to NIDDM or to OLETF rats. In any case, administration of pioglitazone reproduced these two peaks.

On the determination of energy metabolism, values of RQ were significantly lower in rats treated with pioglitazone than controls. Therefore, the energy consumption derived from fat was significantly higher in rats treated with pioglitazone, compared with controls at both 8 and 24 weeks of age. In a previous report (6), we interpreted that since OLETF rats fed with a control diet manifested NIDDM at 24 weeks of age, the lower RQ value at 24 weeks than 8 weeks of age could be due to insufficient glucose utility because of the relative weakness of insulin bioactivity. However, pioglitazone decreased RQ even at 8 weeks of age (without NIDDM) and further decreased it at 24 weeks of age.

PPARγ agonists have been known to influence fat metabolism (12–14). PPARγ was preferentially expressed in adipose tissue and appears to play key roles in the storage of fatty acids. PPARγ agonists as well as insulin in an adipose tissue have adipogenic action (23, 24). We observed in the present study that fat derived energy used for combustion was significantly increased by pioglitazone at both 8 and 24 weeks of age; however, the plasma levels of triglyceride and cholesterol were not different between treatments. Administration of pioglitazone did not influence food intake, body weight or visceral fat weight. Therefore, it is interpreted that pioglitazone increased both adipogenesis and fat combustion; that is, pioglitazone increased metabolic turnover of fat. However, as energy derived from fat did not constitute the rhythm and enhanced fat metabolism has been substantial at 8 weeks of age, the change in fat metabolism does not seem to have an essential role for recovery of two peaks of daily profiles of energy expenditure.

Recent studies (25–27) have shown that adipose cells secrete a large number of bioactive molecules, including adipins, angiotensinogen, TNF-α, leptin and plasminogen activator inhibitor 1, and these factors have been known to interfere with insulin bioactivity. It is reported (27) that troglitazone (another PPARγ agonist) decreased both leptin and TNF-α gene expressions in fat tissues. The plasma level of leptin was not decreased in the present study. This discrepancy may be due to the lower dose of pioglitazone administered in our study. On the other hand, the plasma level of TNF-α was significantly decreased, and reached the normal level, and the levels of fasting blood glucose and HbA1c were decreased, although these did not fully return to the normal levels. The pancreatic histological findings as well as plasma levels of insulin were not different between treatments. Therefore, it is suggested that the decrease in TNF-α by administration of pioglitazone increased insulin sensitivity and glucose utility, and it improved the diabetic condition in terms of decreases in blood HbA1c and in fasting blood glucose level (28). Improvement of the diabetic condition might influence the daily profile of energy expenditure, although it is unknown whether TNF-α directly affected the daily profile of energy expenditure or not. We did not determine the expression of TNF-α in adipose tissue, so it is unknown whether pioglitazone inhibited TNF-α production of circulating monocytes (29) or of adipocytes.

In conclusion, a PPARγ agonist pioglitazone improved the diabetic state via decreasing TNF-α and increased metabolic turnover of fat. It is suggested that administration of pioglitazone improved daily profiles of energy expenditure via altering glucose metabolism as well as fat metabolism.

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